

Reconsolidation and extinction of an appetitive pavlovian memory

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DOI:

[10.1016/j.nlm.2013.04.009](https://doi.org/10.1016/j.nlm.2013.04.009)

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Document Version

Peer reviewed version

Citation for published version (Harvard):

Flavell, CR & Lee, JLC 2013, 'Reconsolidation and extinction of an appetitive pavlovian memory', *Neurobiology of Learning and Memory*, vol. 104C, pp. 25-31. <https://doi.org/10.1016/j.nlm.2013.04.009>

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Reconsolidation and Extinction of an Appetitive Pavlovian Memory

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Running title: Reconsolidation and extinction of appetitive memory

Pages: 18

Figures: 3

Tables: 3

Grant Sponsor: UK Medical Research Council; Grant Number: G0700991

Key Words: NMDA receptor, MK-801, instrumental, food-seeking

Abstract

When memories are retrieved, they can enter a labile state during which the memory may be modified and subsequently restabilized through the process of reconsolidation. However, this does not occur in all situations, and certain “boundary conditions” determine whether a memory will undergo reconsolidation. Naïve male lister hooded rats were trained for 5 days to press a lever in order to retrieve a food reward associated with a pavlovian light stimulus. Three days post-training, animals were injected with either MK-801 (0.1mg.kg⁻¹; i.p.) or saline vehicle, 30 minutes before they were placed back into the training context for a retrieval session. Lever pressing was reinforced only by the light stimulus and was restricted to either 10, 30 or 50 presentations of the light conditioned stimulus. After 48 hours, animals were again returned to the boxes and light-reinforced lever-pressing activity was recorded. MK-801-treated animals in the 10CS group significantly reduced lever pressing at test, compared to saline controls. In contrast, MK-801-treated rats in the 50CS group demonstrated a significant increase. There was no effect of MK-801 in the 30CS group. Additionally, there were no effects of MK-801 in an analogous, pure instrumental, setting when the cue lights were omitted. The opposing effects of MK-801 under different parametric conditions likely reflect impairments of appetitive pavlovian memory reconsolidation and extinction, respectively. These results demonstrate a competition between reconsolidation and extinction. However, there are also conditions under which MK-801 fails to impair either process.

1. Introduction

Reconsolidation is the process proposed to occur that re-stabilizes a memory that has been reactivated through retrieval (Lewis *et al.*, 1972; Nader *et al.*, 2000; Nader, 2003; Finnle & Nader, 2012). If this reconsolidation process is interrupted, memories are prevented from returning to a stable state resulting in long-lasting amnesia. This reactivation-dependent amnesia has been demonstrated across a number of species (e.g. Lewis *et al.*, 1972; Nader *et al.*, 2000; Debiec *et al.*, 2002; Pedreira & Maldonado, 2003; Eisenberg & Dudai, 2004; Pedreira *et al.*, 2004; Rose & Rankin, 2006; Achterberg *et al.*, 2012), including humans (Forcato *et al.*, 2007; Hubbach *et al.*, 2007; Brunet *et al.*, 2008; Kindt *et al.*, 2009), in both appetitive (Lee & Everitt, 2008a; b; c; Flavell *et al.*, 2011; Achterberg *et al.*, 2012) and aversive settings (Nader *et al.*, 2000; Debiec *et al.*, 2002). Moreover, under certain conditions it is possible to enhance (Debiec *et al.*, 2011; Tian *et al.*, 2011) and even incorporate new information (Choi *et al.*, 2010; Lee, 2010) into existing memories, leading to the suggestion that the process of destabilization and subsequent reconsolidation is a mechanism which allows the memory updating required for learning (for review see Lee, 2009).

In pavlovian conditioning settings, memory destabilization is generally achieved by re-exposure to the conditioned stimulus (CS) in the absence of the previously-associated unconditioned stimulus (US). However, the presentation of the CS alone is operationally a short extinction session and could lead to either a reconsolidation of the existing trace or the formation of a new extinction (CS-No US) memory. The conditions in which these two opposing outcomes occur are dictated by several important factors, or boundary conditions (Lee, 2009; Nader & Hardt, 2009). In particular, the balance between the strength of training and the extent of non-reinforced CS exposure appears to determine which of reconsolidation and extinction occurs in aversive pavlovian conditioning settings. When training is kept constant, several reactivation parameters have been identified as important. However, two appear to be critical; first the presentation of new information during the reactivation session (Morris *et al.*, 2006; Lee & Everitt, 2008b; Winters *et al.*, 2009) and second, altering the duration of stimulus re-exposure, as increasing exposure to the CS during reactivation increases the likelihood of extinction rather than reconsolidation being impaired (Pedreira & Maldonado, 2003; Suzuki *et al.*, 2004; Power *et al.*, 2006). Varying the strength of training, while keeping reactivation parameters constant, also impacts upon whether a memory will undergo reconsolidation, with the suggestion that “stronger” memories are harder to destabilize (Eisenberg *et al.*, 2003; Suzuki *et al.*, 2004; Wang *et al.*, 2009; Winters *et al.*, 2009; Reichelt & Lee, 2012). At the present time, no studies have systematically examined the effect of manipulating the extent of CS re-exposure within an appetitive pavlovian setting.

The previous demonstrations of a relationship between reconsolidation and extinction in rodent settings have been achieved by the administration either of a protein synthesis inhibitor administered intracerebrally (Eisenberg *et al.*, 2003) or systemically (Suzuki *et al.*, 2004) or by the systemic injection of the NMDA receptor (NMDAR) antagonist MK-801 (Lee & Everitt, 2008a). Although there are mechanistic differences between reconsolidation and extinction at the cellular level (de la Fuente *et al.*, 2011), both processes are NMDAR-dependent in appetitive pavlovian memory settings (Feltenstein & See, 2007; Lee & Everitt, 2008a; Milton *et al.*, 2008a; Holahan *et al.*, 2010). Therefore, in the present study, we have used systemic injections of MK-801 to determine the behavioural conditions under which the reconsolidation and extinction of an appetitive pavlovian memory occur.

2. Methods

2.1. Subjects

Experimentally-naïve male lister hooded rats (n=75) weighing 220-250g at the beginning of the experiment, were housed in groups of 4 in a holding room maintained at 21°C on a 12 hour light/dark cycle (lights on at 07:00). Once acclimatised to the holding room (48-72 hours), access to food was restricted to 15g standard rat chow, per rat, per day. Water was available *ad libitum* throughout, except during behavioural sessions. All procedures complied with the UK 1986 Animals (Scientific Procedures) Act, and performed under project licence PPL 40/3205. Animals were placed into one of 5 groups, based on the nature of training and the type of retrieval session they received (See Table 1).

Group	Training	Retrieval	n
10CS	5 days	10CS	15
30CS	5 days	30CS	14
50CS	5 days	50CS	16
10press	5 days/no CS	10 lever presses/no CS	14
50press	5 days/no CS	50 lever presses/no CS	16

Table 1. Summary of experimental groups that vary in the length and nature of both training and retrieval. The total numbers of subjects (both saline- and MK-801-administered) for each group are given.

2.2. Behavioural apparatus

All behavioural training and testing took place in eight operant chambers (MedAssociates, Vermont), each measuring 25 cm × 32 cm × 25.5 cm, housed within a sound-attenuating chamber. Two sides of the operant chambers were constructed of steel, while the ceiling, front and back walls were Perspex, the front also serving as a door. On the side walls were located several modules (retractable levers, LED stimulus lights, food magazine, and auditory stimulus generators). The auditory stimulus generators were not used during any behavioural procedure. The grid floors consisted of 19 stainless steel rods (4.8 mm diameter; 1.6 mm from centre-to-centre). The interior of each box was cleaned with a 70% ethanol solution after each subject and below the grid floor was a removable tray, which was also cleaned between animals.

2.3. Behavioural procedures

2.3.1. Training

Rats received 5 days of training. At the beginning of training, subjects were placed into the chambers and the start of the procedure was indicated by illumination of the house light and the presentation

of two levers. In the CS groups, depressing the active lever (pseudo-randomly assigned as left or right, counterbalanced across groups) resulted in the houselight being extinguished, both levers being retracted and the delivery of a sucrose pellet to the food magazine, as well as a 10-second illumination of a CS light above the active lever. In the instrumental (10press and 50press) groups, an active lever press resulted in the retraction of the levers and the delivery of a pellet, but the houselight remained on and no CS light was presented. Ten seconds after pressing the active lever, a new trial was signalled by both levers being presented again and (in the CS groups only) the re-illumination of the house light. There were no contingent outcomes when depressing the inactive lever. This repeated until there had been 30 active lever presses or until 30 minutes had elapsed, whichever was soonest.

2.3.2. Retrieval

Three days after the last training session, rats were placed back into the training context, with both levers presented and the house light on. An active lever press had the same outcome as in training except that it was no longer reinforced with sucrose pellet delivery. Dependent upon the treatment group, sessions were restricted to a maximum of 15 minutes or until either 10, 30 or 50 active lever presses had occurred. Subjects received the non-competitive N-methyl-D-aspartic acid (NMDA) receptor antagonist (+)-MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate] (0.1 mg/kg; 0.1 mg/ml) or sterile saline, intraperitoneally, 30 minutes before the beginning of the session. The dose and timing of the injection is the same as used in our previous study of reconsolidation and extinction in conditioned fear (Lee *et al.*, 2006b).

2.3.3. Test

Forty-eight hours after retrieval, animals were returned to the operant chambers for a 30-minute test session, in which no limit was imposed upon the possible number of active lever presses. Again, the session commenced with the extension of both levers and illumination of the house light. Active lever presses in the CS groups resulted in the illumination of the CS light above the active lever and extinction of the houselight for 1 second, because brief presentations of a pavlovian CS are optimal for it to act as a conditioned reinforcer (Mackintosh, 1974), during which the levers remained inserted in the chamber.

2.4. Statistical methods

Data are presented as mean \pm s.e.m. active and inactive lever presses at test, total numbers of CS–pellet pairings (or pellet deliveries) during training, and number of unreinforced CS presentations (or

lever presses) at memory retrieval. The data from the 30-min test are presented in three 10-min bins, as responding on the active lever declined substantially during the course of the session. Data were checked for consistency and rats were excluded if they were statistical outliers (lying more than 2 standard deviations from the group mean) during training, memory retrieval or test. The data were analysed (PASW Statistics 18 software) using repeated measures ANOVA with factors lever (active vs. inactive), condition (retrieval condition), group (saline vs. MK-801) and bin (1, 2 & 3) as appropriate. Therefore, the data were checked for sphericity, and the Greenhouse-Geisser correction was applied as appropriate. All retrieval conditions for a given training condition were analysed together, followed by exploration of simple effects. Planned comparisons were conducted for individual retrieval conditions even when there were no significant interactions in the initial analysis. Tukey's multiple comparison test was selected for *post hoc* analyses, and a significance level of $p < 0.05$ was selected for all analyses.

3. Results

3.1. Pavlovian CS groups

After 5 days of training to lever press for sucrose and an associated light, the light–sucrose association was retrieved by allowing the rats to press the lever to receive up to 10, 30 or 50 light presentations in the absence of sucrose primary reinforcement (Fig. 1). An overall analysis of all conditions revealed that the effect of MK-801 upon discriminated lever pressing was dependent upon the retrieval condition (lever x bin x condition x group: $F(2.9,57.1)=2.99$, $p=0.04$; lever x condition x group: $F(2,39)=4.55$, $p=0.02$). Further analysis of simple effects revealed that there was an effect of MK-801 in the 10-CS and 50-CS conditions, but not in the 30-CS condition.

In the 10-CS condition, systemic injection of MK-801 resulted in significantly impaired light CS-reinforced lever pressing at the subsequent retention test (Fig. 1A). There was a significant lever x group interaction ($F(1,13)=7.12$, $p=0.02$), with no lever x group x bin interaction ($F(1.6,20.3)=0.22$, $p=0.75$) or main effect of group ($F(1,13)=0.32$, $p=0.58$), indicating that MK-801 impaired discriminated responding on the active lever. Given the lever x group interaction, the simple effects of MK-801 upon active and inactive lever pressing separately were conducted using the pooled error term from the factorial analysis. This revealed an effect of MK-801 on the level of total active lever responding throughout the session ($F(1,23.2)=11.86$, $p=0.002$), while leaving inactive lever responding unaffected ($F(1,23.2)=3.38$, $p=0.08$). The effect of MK-801 in the 10-CS condition was not a result of pre-existing differences as the number of CS–sucrose pairings was matched during training (Saline = 133.4 ± 5.5 , MK-801 = 130.0 ± 7.2 ; $F(1,13)=0.15$, $p=0.70$) and all rats received the maximum of 10 CS presentations at memory retrieval.

In the 30-CS condition, MK-801 injection prior to memory retrieval had no significant impact upon subsequent behavioural at test (Fig. 1B). There were no significant lever x group ($F(1,12)=1.05$, $p=0.33$) or lever x group x bin ($F(2,24)=0.30$, $p=0.74$) interactions, nor was there a significant main effect of group ($F(1,12)=1.69$, $p=0.22$). Again, these observations were not impacted upon by pre-existing group differences, as there were no differences during training (total number of CS–sucrose pairings: Saline = 130.6 ± 6.2 , MK-801 = 132.0 ± 6.4 ; $F(1,12)=1.08$, $p=0.88$) or at memory retrieval (number of CS presentations: Saline = 28.3 ± 1.2 , MK-801 = 30.0 ± 0.0 ; $F(1,12)=1.89$, $p=0.20$).

In the 50-CS condition, MK-801 injection prior to memory retrieval did affect subsequent behaviour at test, with MK-801-treated rats performing at a higher rate than saline-injected controls (Fig. 1C). While there was no lever x group interaction ($F(1,14)=2.93$, $p=0.11$) or main effect of group ($F(1,14)=3.01$, $p=0.11$), there was a significant lever x group x bin interaction ($F(1.8,16.5)=5.00$,

$p=0.04$). Analysis of simple main effects revealed a lever \times group interaction during the first bin ($F(1,14)=4.88$, $p=0.04$). There was also a main effect of group ($F(1,14)=5.17$, $p=0.04$), and further analysis of simple main effects using the pooled error term from the factorial analysis revealed group differences during the first bin for the active ($F(1,28.0)=10.0$, $p=0.004$), but not the inactive ($F(1,28.0)=0.005$, $p=0.94$), lever. In contrast, there were no main effects of group or lever \times group interactions for the 2nd and 3rd bins (F 's < 1.25 , p 's > 0.28). The effect of MK-801 in the first bin was not due to pre-existing differences as the number of CS–sucrose pairings was matched during training (Saline = 128.6 ± 7.7 , MK-801 = 132.1 ± 5.8 ; $F(1,14)=0.15$, $p=0.70$) and there were no differences in the number of unreinforced CS presentations at memory retrieval (number of CS presentations: Saline = 48.5 ± 1.6 , MK-801 = 50.0 ± 0.0 ; $F(1,14)=1.00$, $p=0.33$).

Finally, in a separate group of rats, we conducted a no-retrieval condition, in which Saline or MK-801 were injected in the absence of any memory retrieval (Fig. 2). In this condition, MK-801 injection had no significant impact upon subsequent behavioural performance at test. There were no significant lever \times group ($F(1,14)=0.98$, $p=0.34$) or lever \times group \times bin ($F(1.3,17.6)=0.38$, $p=0.59$) interactions, nor was there a significant main effect of group ($F(1,14)=0.31$, $p=0.59$). Again, these observations were not impacted upon by pre-existing group differences, as there were no differences in CS–US pairings during training (total number of CS–sucrose pairings: Saline = 125.4 ± 5.6 , MK-801 = 125.6 ± 7.3 ; $F(1,14)=0.001$, $p=0.98$).

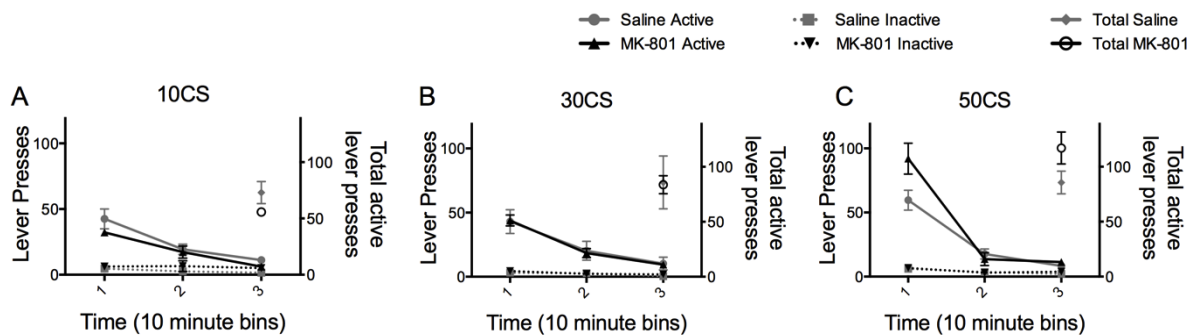


Figure 1. CS-reinforced lever pressing at test in groups, for which the retrieval session was limited to 10 (A), 30 (B) or 50 (C) presentations. The left Y axis shows the number of lever presses per 10-min bin during the test. The total number of active lever presses over the entire 30-minute session is represented on the right Y axis. MK-801-treated animals exhibited a significant decrease in active lever pressing across the test session in the 10CS group compared to saline controls, while MK-801 treated animals in the 50CS group significantly increased active lever responding, selectively in the first bin. There was no effect of MK-801 in the 30CS condition. Data presented as mean \pm S.E.M.

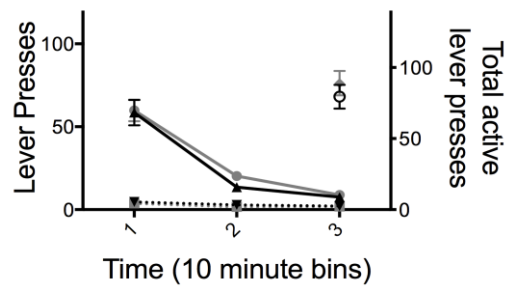


Figure 2 CS-reinforced lever pressing at test in the no-retrieval condition. The left Y axis shows the number of lever presses per 10-min bin during the test. The total number of active lever presses over the entire 30-minute session is represented on the right Y axis. There were no significant differences in active lever pressing across the test session . Data presented as mean \pm S.E.M.

3.2. Instrumental groups

After 5 days of training to lever press for sucrose in the absence of the explicit light stimulus, the instrumental memory was retrieved by allowing the rats to press the lever up to 10 or 50 times (Fig 3A and 3B, respectively). This procedure matched those that had previously revealed behavioural effects of MK-801 with the exception that no light stimulus was present at any time during training, memory retrieval and test. While the lever retraction and sound of the pellet dispenser would be expected to condition classically to sucrose, these stimuli are not present at the test and so lever responding at test primarily assesses the strength of the instrumental associations. Therefore, this procedure helps to disambiguate whether the previously-observed effects were due to impacts upon pavlovian or instrumental memory processes. Systemic injection of MK-801 30 min prior to memory retrieval had no significant effect upon lever pressing at the subsequent retention test in either of the conditions, as evidenced by an overall analysis of both conditions (lever x bin x condition x group: $F(1.4,35.2)=0.18$, $p=0.75$; lever x condition x group: $F(1,26)=0.25$, $p=0.62$; lever x group: ($F(1,26)=1.81$, $p=0.19$); group: $F(1,26)=0.47$, $p=0.50$)). Planned comparisons confirmed that there was no effect of MK-801 under either retrieval condition. There were no lever x group (10 press: $F(1,12)=1.12$, $p=0.31$; 50 press: $F(1,14)=0.60$, $p=0.45$) or lever x group x bin interactions (10 press: $F(1.4,16.8)=0.98$, $p=0.37$; 50 press: $F(1.2,17.0)=0.66$, $p=0.46$), nor were there any main effects of group (10 press: $F(1,12)=0.12$, $p=0.74$; 50 press: $F(1,14)=0.41$, $p=0.53$). These observations were not impacted upon by pre-existing group differences prior to the test as behaviour was matched both during training and at memory retrieval (Table 2).

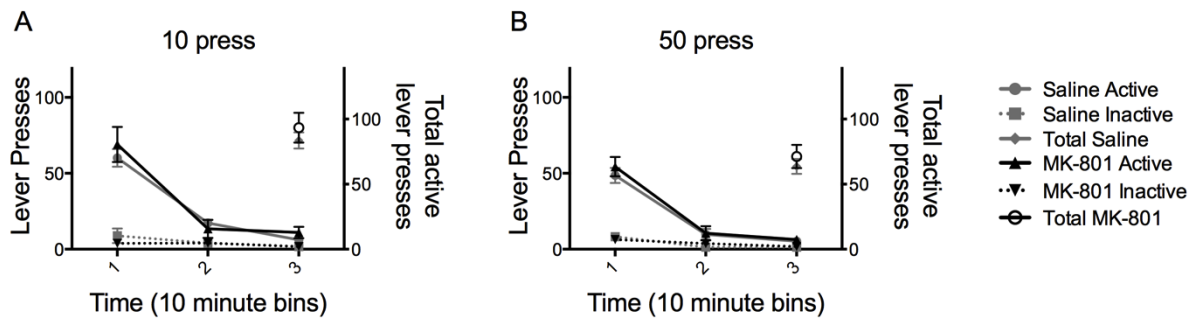


Figure 3. Lever pressing at test after 5 days training in a pure instrumental setting. The left Y axis displays the mean number of lever presses during each 10-min bin of the test, when the retrieval session was limited to either 10 (A) or 50 (B) lever presses. The total number of active lever presses over the entire 30 minute session is plotted on the right Y axis. There were no statistically significant differences between MK-801 treated animals and controls in either condition. Data presented as mean + S.E.M.

Condition	Training/Retrieval	Saline	MK-801	Statistics
10-press	Training	129.7 ± 7.9	125.1 ± 5.5	F(1,12)=0.222, p=0.646
	Retrieval	10	10	n/a
50-press	Training	128.0 ± 6.4	108.0 ± 9.0	F(1,14)=3.438, p=0.085
	Retrieval	46.3 ± 2.3	50.0 ± 0.0	F(1,14)=3.156, p=0.097

Table 2. Performance during training and retrieval of the groups that received 5 days of training without CS presentations. Data are presented as mean ± S.E.M., and were analysed by one-way ANOVA. There were no differences between the saline and MK-801 groups under either of the retrieval conditions.

4. Discussion

Using systemic administration of the NMDAR antagonist MK-801 during memory retrieval, the present study has been able to investigate the effect of retrieval duration on determining whether appetitive pavlovian memories enter a state of reconsolidation or extinction. After 5 days of training, animals that received MK-801 and 10 CS presentations at the retrieval session demonstrated impaired CS-reinforced lever pressing, compared to saline treated controls, at a retention test performed 48 hours later. By contrast, when retrieval consisted of up to 50 CS presentations, MK-801 treated animals displayed a marked increase in lever pressing compared to control. However, with an intermediate retrieval session of up to 30 CS presentations, there were no significant differences between MK-801 and saline treated controls. The impact of MK-801 upon behaviour appears to be related to modulation of the pavlovian CS–sucrose memory, as there were no effects of MK-801 in a parametrically-equivalent instrumental only setting (where no CS cues were given at test).

The effect of MK-801 on the extinction of the CS–sucrose memory is consistent with an extensive literature implicating NMDARs in pavlovian memory extinction. The administration of NMDAR antagonists has previously been shown to impair the consolidation of extinction in aversive pavlovian fear conditioning settings (Santini *et al.*, 2001; Lee *et al.*, 2006b; Burgos-Robles *et al.*, 2007; Liu *et al.*, 2009). In appetitive pavlovian tasks, NMDAR antagonists administered prior to extinction resulted in elevated stimulus-reinforced lever pressing behavior, consistent with impaired extinction (Feltenstein & See, 2007; Kelamangalath *et al.*, 2007; Holahan *et al.*, 2010; Holahan *et al.*, 2012). Importantly, the effect of MK-801 to elevate subsequent CS-reinforced responding was only observed in the 50CS condition, and not in the no-retrieval condition, the 30CS (no effect of MK-801) or 10CS (opposite effect of MK-801) conditions. Therefore, the increase in responding cannot be attributed to a non-specific effect of MK-801, but rather is a result of the direct impact of MK-801 upon processes induced by the CS re-exposure at memory retrieval. The 50CS condition in the present study had the greatest number of CS–noUS pairings, making it the most likely to induce extinction. However, this was not explicit from the levels of responding in the saline control groups across the various conditions. In fact, there was surprisingly no evidence that retrieval with 50 unreinforced CS presentations led to any decline in responding at test compared to any of the other groups, including the no-retrieval condition. Given that the effects of MK-801 and other NMDAR antagonists are assumed to be memory-impairing, rather than memory-enhancing, the most likely interpretation of the current results is that MK-801 did impair memory extinction. It is highly unlikely that in the present setting MK-801 enhanced reconsolidation to elevate responding at test, especially given that the NMDAR partial agonist D-cycloserine (DCS) enhances memory

reconsolidation in both fear and appetitive pavlovian settings (Lee *et al.*, 2006b; a; Lee *et al.*, 2009). Thus the absolute levels of responding observed across the different test conditions may be most likely explained by differences in cohorts of animals.

The effect observed in the 50CS condition appears to be a pavlovian, as there was no effect in the equivalent 50-press instrumental experiment. While it is possible that the extinction of any pavlovian association between sucrose and the retraction of the lever or the sound of the pellet dispenser might have been disrupted by MK-801 at memory retrieval, this would not have had any effect on responding at the test session, in which the levers were never retracted and the pellet dispenser was never activated. Therefore, the equivalence of responding in the saline and MK-801 groups strongly indicates that MK-801 failed to impair instrumental memory extinction under the present conditions. This result is somewhat surprising, given that others have shown that administration of NMDAR antagonists during instrumental tasks will impair both acquisition and extinction (Lissek & Gunturkun, 2003; Yin *et al.*, 2005). However, it is not without precedent, as a study that tested the effects of systemic administration of DCS, at doses known to facilitate pavlovian extinction, reported no enhancements of extinction in a purely operant task (Vurbic *et al.*, 2011). The findings of the present study and those of Vurbic *et al.* (2011) do not conclusively demonstrate that NMDAR modulation does not affect instrumental extinction; rather they indicate that NMDAR modulators appear not to alter instrumental extinction under conditions that engage pavlovian extinction.

The effect of MK-801 to reduce CS-reinforced responding in the 10CS condition is also consistent with literature demonstrating the functional role of NMDARs in memory reconsolidation. Systemic NMDAR antagonism impaired pavlovian fear memories in both auditory (Lee *et al.*, 2006b) and contextual (Suzuki *et al.*, 2004) paradigms. Moreover, there have been several demonstrations that MK-801 impairs memory reconsolidation in a reactivation-dependent manner in appetitive memory settings (Lee *et al.*, 2006b; Lee & Everitt, 2008c; a; Milton *et al.*, 2008a; Milton *et al.*, 2008b; von der Goltz *et al.*, 2009). Importantly, the effect of MK-801 to reduce responding at test was acutely dependent upon the specific parameters at memory retrieval. In the no-retrieval and 30-CS conditions, MK-801 was without effect, and MK-801 elevated subsequent responding when administered in conjunction with the 50-CS retrieval session. Therefore, the performance at test cannot be attributed to a non-specific effect of MK-801. Rather, it is highly likely that it corresponds to an impairment in reconsolidation. Again, it is highly unlikely that MK-801 potentiated memory extinction in the current experiment, especially given that D-cycloserine enhances pavlovian memory extinction in both aversive and appetitive settings (Botreau *et al.*, 2006; Lee *et al.*, 2006b). Moreover, the reconsolidation impairment appears to be specific to the pavlovian memory

component of the task, given that no effect of MK-801 was observed in the parametrically equivalent instrumental experiment. We have previously observed that disruption of pavlovian memory reconsolidation takes place in parallel with preservation of the instrumental memory (Lee *et al.*, 2006b; a; Lee & Everitt, 2008c; Milton *et al.*, 2008b). Moreover, there has yet to be any demonstration that instrumental responding can be diminished through impairment of reconsolidation of the underlying associative memories (Hernandez & Kelley, 2005).

The balance between reconsolidation and extinction in the present study replicates a pattern of results previously observed in aversive conditioning settings. It has previously been demonstrated that amnestic treatment impairs reconsolidation when retrieval is short, whereas it disrupts extinction when retrieval is long (Pedreira & Maldonado, 2003; Suzuki *et al.*, 2004; but see Duvarci *et al.*, 2006). Moreover, we have also shown a similar effect using MK-801 in pavlovian fear conditioning (Lee *et al.*, 2006b). The present results are novel firstly in that they extend this observation to an appetitive pavlovian setting. They also suggest that the balance between the effect of MK-801 on reconsolidation and extinction is related to the retrieval condition directly, rather than the behavioural impact of that session, given that there is no obvious extinction of responding in the 50CS condition compared to the 10CS condition in the present data. Finally, the absence of any behavioural impact of MK-801 in the 30CS condition indicates that the balance between reconsolidation and extinction is more complex than previously thought.

In the 30CS condition MK-801 appeared to impair neither reconsolidation nor extinction, leaving performance at test unaffected. Previous interpretations of the balance between reconsolidation and extinction have argued that reconsolidation mechanisms are dominant until the length of reactivation becomes sufficient to engage extinction, and it has been postulated that the dominant trace is the one that will be left vulnerable to amnestic drug treatments (Eisenberg *et al.*, 2003). Therefore, the functional engagement of extinction appears to be a boundary condition upon reconsolidation (Lee, 2009; Nader & Hardt, 2009). However, the present lack of effect observed with an intermediate length of retrieval in the 30CS condition may suggest that it is not extinction *per se* that exerts the boundary effect. Instead of there being a competition between reconsolidation and extinction, the conditions under which reconsolidation take place may be defined independently of the functional engagement of extinction. We have previously argued that the boundary conditions on reconsolidation might be unified under the concept of memory updating, with new experiences either updating the existing memory via reconsolidation or supplementing it with new learning (Lee, 2009). Under such a scheme, extinction learning would be expected to comprise new learning and hence suppress memory reconsolidation. Indeed, in the hippocampus, a cellular mechanism for the

suppression of reconsolidation by extinction, comprising the inhibition of nuclear factor- κ B (NF- κ B) by calcineurin has been identified (de la Fuente *et al.*, 2011). Therefore, it remains unclear whether the present results in the 30CS condition represent a molecular switching point at which reconsolidation is suppressed, but extinction is not yet functionally engaged to a level at which disruption can be detected behaviourally. Moreover, it will be important to determine whether the present intermediate point in memory retrieval can be replicated in aversive conditioning settings.

Taken together, these data show that the number of CS re-exposures is an important boundary condition that determines whether reconsolidation or extinction will occur. Moreover, there is more than a simple competition between reconsolidation and extinction, with there being behavioural conditions under which MK-801 has no impact upon subsequent stimulus-supported responding. Therefore, attempts to modulate reconsolidation or extinction in appetitive reward-seeking settings must carefully consider the parameters of memory retrieval used to reactivate the pavlovian memory.

5. References

- Achterberg, E.J., Trezza, V. & Vanderschuren, L.J. (2012) beta-Adrenoreceptor Stimulation Mediates Reconsolidation of Social Reward-Related Memories. *PloS one*, **7**, e39639.
- Botreau, F., Paolone, G. & Stewart, J. (2006) d-Cycloserine facilitates extinction of a cocaine-induced conditioned place preference. *Behav Brain Res*, **172**, 173-178.
- Brunet, A., Orr, S.P., Tremblay, J., Robertson, K., Nader, K. & Pitman, R.K. (2008) Effect of post-retrieval propranolol on psychophysiologic responding during subsequent script-driven traumatic imagery in post-traumatic stress disorder. *J Psychiatr Res*, **42**, 503-506.
- Burgos-Robles, A., Vidal-Gonzalez, I., Santini, E. & Quirk, G.J. (2007) Consolidation of Fear Extinction Requires NMDA Receptor-Dependent Bursting in the Ventromedial Prefrontal Cortex. *Neuron*, **53**, 871-880.
- Choi, J.H., Kim, J.E. & Kaang, B.K. (2010) Protein synthesis and degradation are required for the incorporation of modified information into the pre-existing object-location memory. *Molecular brain*, **3**, 1.
- de la Fuente, V., Freudenthal, R. & Romano, A. (2011) Reconsolidation or extinction: transcription factor switch in the determination of memory course after retrieval. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **31**, 5562-5573.
- Debiec, J., Bush, D.E. & LeDoux, J.E. (2011) Noradrenergic enhancement of reconsolidation in the amygdala impairs extinction of conditioned fear in rats--a possible mechanism for the persistence of traumatic memories in PTSD. *Depression and anxiety*, **28**, 186-193.
- Debiec, J., LeDoux, J.E. & Nader, K. (2002) Cellular and systems reconsolidation in the hippocampus. *Neuron*, **36**, 527-538.
- Duvarci, S., Mamou, C.B. & Nader, K. (2006) Extinction is not a sufficient condition to prevent fear memories from undergoing reconsolidation in the basolateral amygdala. *Eur J Neurosci*, **24**, 249-260.
- Eisenberg, M. & Dudai, Y. (2004) Reconsolidation of fresh, remote, and extinguished fear memory in Medaka: old fears don't die. *The European journal of neuroscience*, **20**, 3397-3403.
- Eisenberg, M., Kobil, T., Berman, D.E. & Dudai, Y. (2003) Stability of retrieved memory: Inverse correlation with trace dominance. *Science*, **301**, 1102-1104.
- Feltenstein, M.W. & See, R.E. (2007) NMDA receptor blockade in the basolateral amygdala disrupts consolidation of stimulus-reward memory and extinction learning during reinstatement of cocaine-seeking in an animal model of relapse. *Neurobiol Learn Mem*, **88**, 435-444.
- Finnie, P.S. & Nader, K. (2012) The role of metaplasticity mechanisms in regulating memory destabilization and reconsolidation. *Neuroscience and biobehavioral reviews*.
- Flavell, C.R., Barber, D.J. & Lee, J.L.C. (2011) Behavioural memory reconsolidation of food and fear memories. *Nat Commun*, **2**, 504.
- Forcato, C., Burgos, V.L., Argibay, P.F., Molina, V.A., Pedreira, M.E. & Maldonado, H. (2007) Reconsolidation of declarative memory in humans. *Learn Mem*, **14**, 295-303.
- Hernandez, P.J. & Kelley, A.E. (2005) Cracking addiction the second time around: reconsolidation of drug-related memories. *Neuron*, **47**, 772-775.
- Holahan, M.R., Clarke, M.J. & Hines, D.D. (2010) Dopamine-mediated MK-801-induced elevation in food-based extinction responding in rats and associated changes in region-specific phosphorylated ERK. *Psychopharmacology*, **212**, 393-403.
- Holahan, M.R., Westby, E.P. & Albert, K. (2012) Comparison of the MK-801-induced appetitive extinction deficit with pressing for reward and associated pERK1/2 staining in prefrontal cortex and nucleus accumbens. *Behavioural brain research*, **228**, 194-202.
- Hupbach, A., Gomez, R., Hardt, O. & Nadel, L. (2007) Reconsolidation of episodic memories: A subtle reminder triggers integration of new information. *Learn Mem*, **14**, 47-53.

- Kelamangalath, L., Swant, J., Stramiello, M. & Wagner, J.J. (2007) The effects of extinction training in reducing the reinstatement of drug-seeking behavior: involvement of NMDA receptors. *Behavioural brain research*, **185**, 119-128.
- Kindt, M., Soeter, M. & Vervliet, B. (2009) Beyond extinction: erasing human fear responses and preventing the return of fear. *Nat Neurosci*, **12**, 256-258.
- Lee, J.L. & Everitt, B.J. (2008a) Appetitive memory reconsolidation depends upon NMDA receptor-mediated neurotransmission. *Neurobiology of learning and memory*, **90**, 147-154.
- Lee, J.L. & Everitt, B.J. (2008b) Reactivation-dependent amnesia for appetitive memories is determined by the contingency of stimulus presentation. *Learn Mem*, **15**, 390-393.
- Lee, J.L. & Everitt, B.J. (2008c) Reactivation-dependent amnesia in Pavlovian approach and instrumental transfer. *Learn Mem*, **15**, 597-602.
- Lee, J.L., Gardner, R.J., Butler, V.J. & Everitt, B.J. (2009) D-cycloserine potentiates the reconsolidation of cocaine-associated memories. *Learn Mem*, **16**, 82-85.
- Lee, J.L., Milton, A.L. & Everitt, B.J. (2006a) Cue-induced cocaine seeking and relapse are reduced by disruption of drug memory reconsolidation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **26**, 5881-5887.
- Lee, J.L., Milton, A.L. & Everitt, B.J. (2006b) Reconsolidation and extinction of conditioned fear: inhibition and potentiation. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **26**, 10051-10056.
- Lee, J.L.C. (2009) Reconsolidation: maintaining memory relevance. *Trends Neurosci*, **32**, 413-420.
- Lee, J.L.C. (2010) Memory reconsolidation mediates the updating of hippocampal memory content. *Front Behav Neurosci*, **4**, 168.
- Lewis, D.J., Bregman, N.J. & Mahan, J.J., Jr. (1972) Cue-dependent amnesia in rats. *Journal of comparative and physiological psychology*, **81**, 243-247.
- Lissek, S. & Gunturkun, O. (2003) Dissociation of extinction and behavioral disinhibition: the role of NMDA receptors in the pigeon associative forebrain during extinction. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **23**, 8119-8124.
- Liu, J.L., Li, M., Dang, X.R., Wang, Z.H., Rao, Z.R., Wu, S.X., Li, Y.Q. & Wang, W. (2009) A NMDA receptor antagonist, MK-801 impairs consolidating extinction of auditory conditioned fear responses in a Pavlovian model. *PloS one*, **4**, e7548.
- Mackintosh, N.J. (1974) *The psychology of animal learning*. Academic Press, London ; New York.
- Milton, A.L., Lee, J.L., Butler, V.J., Gardner, R. & Everitt, B.J. (2008a) Intra-amygdala and systemic antagonism of NMDA receptors prevents the reconsolidation of drug-associated memory and impairs subsequently both novel and previously acquired drug-seeking behaviors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **28**, 8230-8237.
- Milton, A.L., Lee, J.L. & Everitt, B.J. (2008b) Reconsolidation of appetitive memories for both natural and drug reinforcement is dependent on {beta}-adrenergic receptors. *Learn Mem*, **15**, 88-92.
- Morris, R.G., Inglis, J., Ainge, J.A., Olverman, H.J., Tulloch, J., Dudai, Y. & Kelly, P.A. (2006) Memory Reconsolidation: Sensitivity of Spatial Memory to Inhibition of Protein Synthesis in Dorsal Hippocampus during Encoding and Retrieval. *Neuron*, **50**, 479-489.
- Nader, K. (2003) Memory traces unbound. *Trends in neurosciences*, **26**, 65-72.
- Nader, K. & Hardt, O. (2009) A single standard for memory: the case for reconsolidation. *Nature reviews. Neuroscience*, **10**, 224-234.
- Nader, K., Schafe, G.E. & Le Doux, J.E. (2000) Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature*, **406**, 722-726.
- Pedreira, M.E. & Maldonado, H. (2003) Protein synthesis subserves reconsolidation or extinction depending on reminder duration. *Neuron*, **38**, 863-869.
- Pedreira, M.E., Perez-Cuesta, L.M. & Maldonado, H. (2004) Mismatch between what is expected and what actually occurs triggers memory reconsolidation or extinction. *Learn Mem*, **11**, 579-585.

- Power, A.E., Berlau, D.J., McGaugh, J.L. & Steward, O. (2006) Anisomycin infused into the hippocampus fails to block "reconsolidation" but impairs extinction: The role of re-exposure duration. *Learn Mem*, **13**, 27-34.
- Reichelt, A.C. & Lee, J.L. (2012) Appetitive Pavlovian goal-tracking memories reconsolidate only under specific conditions. *Learn Mem*, **20**, 51-60.
- Rose, J.K. & Rankin, C.H. (2006) Blocking memory reconsolidation reverses memory-associated changes in glutamate receptor expression. *J Neurosci*, **26**, 11582-11587.
- Santini, E., Muller, R.U. & Quirk, G.J. (2001) Consolidation of extinction learning involves transfer from NMDA-independent to NMDA-dependent memory. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **21**, 9009-9017.
- Suzuki, A., Josselyn, S.A., Frankland, P.W., Masushige, S., Silva, A.J. & Kida, S. (2004) Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **24**, 4787-4795.
- Tian, S., Huang, F., Li, P., Li, Z., Zhou, S., Deng, H. & Yang, Y. (2011) Nicotine enhances contextual fear memory reconsolidation in rats. *Neuroscience letters*, **487**, 368-371.
- von der Goltz, C., Vengeliene, V., Bilbao, A., Perreau-Lenz, S., Pawlak, C.R., Kiefer, F. & Spanagel, R. (2009) Cue-induced alcohol-seeking behaviour is reduced by disrupting the reconsolidation of alcohol-related memories. *Psychopharmacology*, **205**, 389-397.
- Vurbic, D., Gold, B. & Bouton, M.E. (2011) Effects of D-cycloserine on the extinction of appetitive operant learning. *Behavioral neuroscience*, **125**, 551-559.
- Wang, S.H., de Oliveira Alvares, L. & Nader, K. (2009) Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. *Nature neuroscience*, **12**, 905-912.
- Winters, B.D., Tucci, M.C. & DaCosta-Furtado, M. (2009) Older and stronger object memories are selectively destabilized by reactivation in the presence of new information. *Learn Mem*, **16**, 545-553.
- Yin, H.H., Knowlton, B.J. & Balleine, B.W. (2005) Blockade of NMDA receptors in the dorsomedial striatum prevents action-outcome learning in instrumental conditioning. *The European journal of neuroscience*, **22**, 505-512.

Figure and Table Legends

Figure 1. CS-reinforced lever pressing at test in groups, for which the retrieval session was limited to 10 (A), 30 (B) or 50 (C) presentations. The left Y axis shows the number of lever presses per 10-min bin during the test. The total number of active lever presses over the entire 30-minute session is represented on the right Y axis. MK-801-treated animals exhibited a significant decrease in active lever pressing across the test session in the 10CS group compared to saline controls, while MK-801 treated animals in the 50CS group significantly increased active lever responding, selectively in the first bin. There was no effect of MK-801 in the 30CS condition. Data presented as mean \pm S.E.M.

Figure 2 CS-reinforced lever pressing at test in the no-retrieval condition. The left Y axis shows the number of lever presses per 10-min bin during the test. The total number of active lever presses over the entire 30-minute session is represented on the right Y axis. There were no significant differences in active lever pressing across the test session . Data presented as mean \pm S.E.M.

Figure 3. Lever pressing at test after 5 days training in a pure instrumental setting. The left Y axis displays the mean number of lever presses during each 10-min bin of the test, when the retrieval session was limited to either 10 (A) or 50 (B) lever presses. The total number of active lever presses over the entire 30 minute session is plotted on the right Y axis. There were no statistically significant differences between MK-801 treated animals and controls in either condition. Data presented as mean + S.E.M.

Table 1. Summary of experimental groups that vary in the length and nature of both training and retrieval. The total numbers of subjects (both saline- and MK-801-administered) for each group are given.

Table 2. Performance during training and retrieval of the groups that received 5 days of training without CS presentations. Data are presented as mean \pm S.E.M., and were analysed by one-way ANOVA. There were no differences between the saline and MK-801 groups under either of the retrieval conditions.